Patent
Attorney's Docket No. 2632-1-001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Pierre Belhumeur et al.
SERIAL NO.:	09/980,649
FILED:	June 4, 2002
FOR:	BIOLOGICAL INDICATORS FOR VALIDATING A PRION STERILIZATION PROCESS
ART UNIT:	1651
EXAMINER:	Taeyoon Kim

DECLARATION UNDER 37 C.F.R. SEC. 1.132

I, Pierre Belhumeur, do hereby declare and state as follows:

1. I received the degrees of Bachelor (B.Sc.) of Microbiology from Laval University (Quebec, Canada) in 1981, Master (M.Sc.) of Microbiology and Immunology from University of Montreal (Montreal, Canada) in 1984, and Doctor of Philosophy (Ph.D.) of Molecular Biology from University of Montreal (Montreal, Canada) in 1989. I am presently a Professor and Director of the microbiology and immunology department at the University of Montreal.
2. My academic background and experiences in the field of the present invention are listed on the enclosed *curriculum vitae*.
3. I am an author of several scholarly publications as listed in my enclosed *curriculum vitae*.
4. I am an inventor in the present application; I have read and am thoroughly familiar with the contents of U.S. Patent Application Serial No. 09/980,649 entitled "BIOLOGICAL INDICATORS FOR VALIDATING A PRION STERILIZATION PROCESS", including the claims presently on file.

5. I have also read and understood the latest Official Action from the PTO dated May 28, 2008. In this Office Action, claims 3 and 5-15 were rejected for being obvious in view of Safar et al., in view of Coustou et al., Glover et al. or Wickner under 35 U.S.C. 103(a). Claim 9 was also rejected for being obvious in view of Safar et al., in view of Wickner, Glover et al., or Coustou et al., in further view of Feldman et al. under 35 U.S.C. 103(a). Claims 9, 10 and 13 were rejected for being obvious in view of Safar et al., in view of Wickner, Glover et al., or Coustou et al., in further view of Dresdner Jr. et al. under 35 U.S.C. 103(a).

6. It is understood that the Examiner is taking the position that Safar et al. teaches the steps of subjecting prion protein in a container to a sterilization process (temperature and/or chemical treatment), and determinating of the level of degradation/inactivation of the prion protein measured by Western blotting. Furthermore, the Examiner is under the impression that yeast prion proteins are known as mammal prion analog possessing the same property as a mammalian counterpart.

7. In this regard, I wish to point out that the goal of the study published by Safar et al. was to study the thermal stability and ***conformational transitions*** of scrapie amyloid protein and its correlation with infectivity. For that, they submitted scrapie amyloid protein to heat treatment and to chemical scrapie inactivators such as FA, SDS, additional α-helix-inducing fluorinated alcohols and TFA. Safar et al. show that the mentioned treatments that do affect the conformation of the scrapie amyloid protein can also affect its infectivity. Indeed, their western blot analysis show that there can be dimer formation of scrapie protein upon some of those treatments.

8. On the contrary, the claimed method of U.S. Patent Application Serial No. 09/980,649 is a method for evaluating the ***efficiency of a sterilization process***, as claimed, and not to measure the conformational transitions of scrapie. Since some sterilization processes allow a significant degradation of prion proteins whereas other methods produce a weaker degradation, the method claimed in the present application allows the evaluation of the efficacy of different sterilization processes. The present application teaches a method that can be adapted to industrial processes wherein there is a need to control the efficiency of the sterilization process. By western blot analysis, the present application shows that there is no residual yeast prion protein detectable after ozone treatment (see for example Table 1 of U.S. Patent Application Serial No. 09/980,649). Ozone therefore goes beyond all the treatments described by Safar as ozone is an extremely powerful oxidative process, able to break down chemical bonds. In that respect, it differs profoundly from Safar where no such observation has been made with their treatments.
9. Thus, the mere fact of knowing, from Safar et al., that heat or chemical treatment can have an effect on the degradation of a prion protein and that the level of degradation can be measured by Western blot analysis is not enough to lead one having ordinary skill in the art to elaborate a method of evaluating the efficiency of a sterilization process.
10. Furthermore, contrary to the Examiner's position that yeast prion proteins are known as mammal prion analog possessing the same property as a mammalian counterpart, a person skilled in the art would agree with me that there are significant differences between yeast prion proteins and mammalian prion proteins. Firstly, there is a low level of homology between the amino acid sequences of mammalian prion proteins and yeast prion protein. Further, the mechanism of conversion of mammalian and yeast prions into

their infectious forms differ significantly. The yeast protein is a cytosolic protein able to undergo the change of conformation at physiological pH while the mammalian counterpart is membrane bound and changes is topology mainly at pH 4.0. In addition, while mammalian prions aggregates are made of fibrils, there is no conclusive evidence that it is also the case for yeast prions. Supporting these affirmations is the article of Bousset and Melki enclosed herewith (Microbes and Infection, 2002, 4: 461-469). These differences are significant enough in order not to allow a person skilled in the art to easily conceive from a method of measuring conformational changes of a mammalian prion a method of measuring the efficiency of a sterilization process.

11. Therefore, a person skilled in the art will acknowledge that the methods taught in the present application are not obvious having regard to Safar et al.

12. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by a fine or imprisonment, or both (18 U.S.C. Sec. 1001), and may jeopardize the validity of the application of any patent issuing thereon.

Signed _____ Dated: Aug 27, 2008
Pierre Belhumeur August 27, 2008

CURRICULUM VITAE

Section I- Identification

Name and first name:	Belhumeur, Pierre
Sex:	Male
Date of birth:	1959-08-12
Home Adress:	30 Alexandre Laval (Québec) H7G 3K9 450-667-8767
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Citizenship:	Canadian (born in Trois-Rivières, Québec, Canada)

Section II- Training and experience

1980	B.Sc. II (Biological Sciences), Université de Montréal, Québec
1981	B.Sc. (Microbiology), Université Laval, Québec
1984	M.Sc. (Microbiology and Immunology), Université de Montréal, Québec Importance du gène *SFIB* dans la division cellulaire d'*Escherichia coli* K12 (Supervisor: Dr. G.R. Drapeau)
1989	Ph.D. (Molecular Biology), Université de Montréal, Québec Expression de transcrits naturels, complémentaires au messager de la protéine ribosomale L27' chez la souris (Supervisor: Dr. D. Skup))
07/89 - 06/90	Postdoctoral training; National Institute for Medical Research, Mill Hill, UK (Dr. Frank Grosveld)
07/90 - 06/92	Postdoctoral training; Biology Dept., McGill University (Montreal, Canada) (Dr. Michael W. Clark)

(Studentships)

1983 - 1984	Cancer Research Society (Montreal, Canada)
1984 - 1989	NCIC (Ph.D.)
1989 - 1992	Postdoctoral fellowship from MRC (Canada)

Section III- Academic career

06/1992 – 05/1998	Assistant Professor, Dept. of Microbiology & Immunology Université de Montréal
06/1998 – 05/2004	Associate Professor, Dept. of Microbiology & Immunology Université de Montréal
06/2004 – present	Full Professor, Dept. of Microbiology & Immunology Université de Montréal
06/2004 – 05/2005	Chairman (interim), Dept. of Microbiology & Immunology Université de Montréal
06/2005 -05/2009	Chairman, Dept. of Microbiology & Immunology Université de Montréal

Section IV- Publications and Communications

A- Publications.

1) Book chapters

Greaves, D.R., Talbot, D., Philipsen, S., Pruzina, S., deBoer, E., Hanscombe, O., Fraser, P., Antoniou, M., Lindenbaum, M., Dillon, N., **Belhumeur, P.**, Stouboulis, J. and Grosveld, F. Studies of the ß-globin gene - creation of models for somatic gene therapy. In: K.E. Davies, ed., Application of Molecular Genetics to the Diagnosis of Inherited Disease. Royal College of Physicians of London, UK: 1990; 77-84.

Skup, D., Haggarty, A., Paterno, G.D., Ponton, A., Daigneault, L. and **Belhumeur, P.** Regulation and role of IFN gene expression in differentiating embryonal carcinoma cells. In: Y. Kawade and S. Kobayashi, eds., The Biology of the Interferon System VI. Amsterdam, Netherland: Elsevier, 1988; 413-419.

2) Peer reviewed publications

Xu Z, Zheng Y, Ao Z, Clément M, Mouland AJ, Kalpana GV, **Belhumeur P**, Cohen EA and Yao XJ Requirement of the chromatin binding region within the C-terminal catalytic core domain of HIV-1 integrase for the yeast lethality and viral replication. Retrovirology (submitted)

Croisetière S, Tarte PD, Bernatchez L and **Belhumeur P.** (2008) Identification of MHC class IIb resistance/susceptibility alleles to Aeromonas salmonicida in brook charr (Salvelinus fontinalis). Mol. Immunol. 45: 3107–3116.

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Dautremepuits, C., Fortier, M., Croisetière, S., **Belhumeur, P.** and Fournier, M. (2006). Modulation of juvenile brook trout (*Salvelinus fontinalis*) cellular immune system after *Aeromonas salmonicida* challenge. Vet. Immunol. Immunopathol. 110: 27-36.

Perry, G.M.L., Tarte, P.D., Croisetière, S., **Belhumeur, P.** and Bernatchez, L. (2004) Genetic variance and covariance for 0+ brook charr (*Salvelinus fontinalis*) weight and survival time of furonculosis (*Aeromonas salmonicida*) exposure. Aquaculture 235: 263-271.

Yao, Y.J., Lemay, J., Rougeau, N., Clément, M., Kurtz, S., **Belhumeur, P.** and Cohen, E.A. (2002) Genetic selection of peptide inhibitors of Human Immunodeficiency Virus Type 1 Vpr. J. Biol. Chem. 277: 48816-48826.

Ripeau, J.S., Aumont, F., **Belhumeur, P.**, Ostrosky-Zeichner, L., Rex, J.H. and de Repentigny, L. (2002) Effect of the echinocandin caspofungin on expression of *Candida albicans* secretory aspartyl proteinases and phospholipase in vitro. Antimicrob. Agents Chemother. 46: 3096-3100.

Ripeau, J.S., Fiorillo, M., Aumont, F., **Belhumeur, P.** and de Repentigny, L. (2002) Evidence for differential expression of *Candida albicans* virulence genes during oral infection in intact and HIV-1 transgenic mice. J. Infect. Dis. 185:1094-1102

Clément, M., Lavallée, F., Barbès-Morin, G., de Repentigny, L. and **Belhumeur, P.** (2001) Overexpression of Bud5p can suppress mutations of the Gsp1p guanine nucleotide exchange factor Prp20p in *Saccharomyces cerevisiae*. Mol. Genet. Genomics 266: 20-27

Marston, A., Chen, T., **Belhumeur, P.** and Chant, J. (2001) A localized GTPase exchange factor, Bud5, determines the orientation of division axes in yeast. Curr. Biol. 11: 803-807

Clément, M., Fournier, H., Ouspenski, I.I., de Repentigny, L. and **Belhumeur, P.** (2001) Molecular cloning and expression of CaYRB1, the *Candida albicans* RanBP1/YRB1 homologue. Yeast 8: 915-922.

Clément, M., Fournier, H., de Repentigny, L. and **Belhumeur, P.** (2000) Characterization of CaGSP1, the *Candida albicans* RAN/GSP1 homologue. Gene 250:159-169.

de Repentigny, L., Aumont, F., Bernard, K. and **Belhumeur, P.** (2000) Characterization of binding of *Candida albicans* to small-intestinal mucin and its role in adherence to mucosal epithelial cells. Infect Immun. 68:3172-3179.

Dubois, N., Colina, A.R., Aumont, F., **Belhumeur, P.** and de Repentigny, L. (1998) Expression of the gene coding for secretory aspartyl proteinase 2 in *Saccharomyces cerevisiae* and its overexpression in *Candida albicans*. Microbiology 144: 2299-2310.

Stochaj, U., Héjazi, M. and **Belhumeur, P.** (1998) The small GTPase Gsp1p binds to the repeat domain of the nucleoporin Nsp1p. Biochem. J. 330: 421-427.

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Colina, A.R., Aumont, F., Deslauriers, N., **Belhumeur, P.** and de Repentigny, L. (1996) Evidence for degradation of gastrointestinal mucin by *Candida albicans* secretory aspartyl proteinase. Infect. Immun. 64: 4514-4519.

Colina, A.R., Aumont, F., **Belhumeur, P.** and de Repentigny, L. (1996) Detection of secretory mucinolytic activity from *Candida albicans*. J. Med. and Vet. Mycol. 34: 401-406.

Belhumeur, P., Fortin, N. and Clark, M.W. (1994) A gene from *Saccharomyces cerevisiae* which codes for a protein with significant homology to the bacterial 3-phosphoserine aminotransferase. Yeast 10: 385-389.

Lee, A., Tam, R., **Belhumeur, P.**, DiPaolo, T. and Clark, M.W. (1993) *PRP20*, the *Saccharomyces cerevisiae* homolog of the regulator of chromosome condensation, *RCC1*, interacts with double-stranded DNA through a multi-component complex containing GTP-binding proteins. J. Cell Sci. 106: 287-298.

Belhumeur, P., Lanoix, J., Blais, Y., Forget, D., Steyaert, A. and Skup, D. (1993) Action of spontaneously produced interferon ß in differentiation of embryonal carcinoma cells through an autoinduction mechanism. Mol. Cell. Biol. 13: 2846-2857.

Belhumeur, P., Lee, A., Tam, R., DiPaolo, T., Fortin, N. and Clark, M.W. (1993) *GSP1* and *GSP2*, genetic suppressors of the *prp20-1* mutant in *Saccharomyces cerevisiae*: GTP-binding proteins involved in the maintenance of nuclear organization. Mol. Cell. Biol. 13: 2152-2161.

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Grosveld, F., Greaves, D., Philipsen, S., Talbot, D., Pruzina, S., deBoer, E., Hanscombe, O., **Belhumeur, P.**, Hurst, J., Fraser, P., Whyatt, D., Antoniou, M., Mignotte, V., Dillon, N., Lindenbaum, M. and Strouboulis, J. (1990) The Dominant Control Region of the Human Beta-Globin Domain. Annals NY Acad. Sci. 612: 152-159.

Fleming, G., **Belhumeur, P.**, Skup, D. and Fried, H.M. (1989) Functional substitution of yeast ribosomal protein L29 by mouse ribosomal protein L27' in yeast ribosomes. Proc. Natl. Acad. Sci. USA 86: 217-221.

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Belhumeur, P., Paterno, G.D., Boileau, G., Claverie, J.-M. and Skup, D. (1987) Isolation and characterisation of a murine cDNA clone highly homologous to the yeast L29 ribosomal protein gene. Nucl. Acids Res. 15: 1019-1029.

Belhumeur, P. and Drapeau, G.R. (1984) Regulation of cell division in *Escherichia coli*: properties of ftsZ mutants. Molec. Gen. Genet. 197: 254-260.

B- Communications.

S. Croisetière, P.D. Tarte, L. Bernatchez and **P. Belhumeur**. Identification of MHC class IIβ resistance/susceptibility alleles to *Aeromonas salmonicida* in brook charr (*Salvelinus fontinalis*). 9th International Symposium on *Aeromonas* and *Plesiomona*, Vila Real, Portugal. 2008.

A. Faille, E. Boucher, D. Proulx, G. Vandenberg and **P. Belhumeur**. Options for the control of *Saprolegnia* sp in freshwater fish species. Aquaculture Association of Canada, Edmonton, Canada. 2007.

J. Shareck, M. Clément and **P. Belhumeur**. Omega-6 fatty acid gamma-linolenic acid inhibits the yeast-to-hypha transition of pathogenic yeast *Candida albicans*. CSM 57th Annual Meeting, Québec, Canada. 2007.

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K. Lainesse, K. Couillard, M. Clément and **P. Belhumeur**. The nuclear GTPase Gsp1p interacts with Lre1p, a potential cAMP pathway effector of *Saccharomyces cerevisiae*. CSM 53rd Annual Meeting, Laval, Canada. 2003.

K. Lainesse, K. Couillard, M. Clément and **P. Belhumeur**. The nuclear GTPase Gsp1p interacts with Lre1p, a potential cAMP pathway effector of *Saccharomyces cerevisiae*. North Eastern Regional Yeast (NERY) Meeting. Montreal, Canada. 2003.

E.A. Cohen, X.-J. Yao, J. Lemay, N. Rougeau, M. Clément, S. Kurtz, and **P. Belhumeur**. Genetic Selection of peptide inhibitors of human immunodeficiency virus type 1 (HIV-1) Vpr. XIV International AIDS Conference. Barcelona, Spain. 2002.

X.-J. Yao, N. Rougeau, M. Clément, S. Kurtz, **P. Belhumeur** and E.A. Cohen. Genetic Selection of peptide inhibitors of HIV-1 Vpr-mediated cell cycle G2 arrest. The First Annual Scientific Meeting of Canadian Vaccine Networks (CANVAC). Calgary, Canada. 2001.

K. Couillard, M. Clément, F. Lavallée and **P. Belhumeur**. Interaction between the nuclear GTPase Gsp1p and the cAMP pathway-associated protein Lre1p. North Eastern Regional Yeast meeting. Toronto, Canada. 2001.

M. Clément, F. Deshaies, L. de Repentigny and **P. Belhumeur**. Interactions between the nuclear GTPase Gsp1p and the telomeric protein Sir4p in S. cerevisiae. North Eastern Regional Yeast meeting. Toronto, Canada. 2001.

J.S. Ripeau, M. Fiorillo, F. Aumont, **P. Belhumeur**, L. de Repentigny. Evidence for differential expression of Candida albicans virulence genes during oral candidiasis. ASM 101th General Meeting. Orlando, USA. 2001.

M. Clément, G. Barbès-Morin, F. Lavallée, L. de Repentigny and **P. Belhumeur**. Bud5p as a potential cytoplasmic guanine exchange factor for Gsp1p. Yeast Genetics and Molecular Biology Meeting. Seattle, USA. 2000.

M. Fiorillo, J.S. Ripeau, F. Aumont, **P. Belhumeur** and L. de Repentigny. Differential Gene Expression in Oral and Vaginal Candidiasis. ASM 100th General Meeting. Los Angeles, USA. 2000.

X.-J. Yao, N. Rougeau, M. Hrimech, M. Clément, S. Kurtz, **P. Belhumeur** and E.A.Cohen. Genetic Selection of Peptide Inhibitors of HIV-1 Vpr-Mediated Cell Cycle G2 Arrest. Novel Biological Approaches to HIV-1 Infection Based on New insights into HIV Biology. Keystone Symposia. Keystone (CO), USA. 2000.

J.S. Ripeau, M. Fiorillo, F. Aumont, **P. Belhumeur** and L. de Repentigny. In vivo expression of Candida albicans virulence determinants in oral candidiasis. ASM Conference on Candida and Candidiasis. Charleston, USA. 2000.

M. Clément, L. de Repentigny and **P. Belhumeur**. New interactions between the telomeric protein Sir4p and the nuclear GTPase Gsp1p in Saccharomyces cerevisiae. Canadian Society of Microbiologists. Montréal, Canada. 1999.

M. Clément, H. Fournier, L. de Repentigny and **P. Belhumeur**. Molecular cloning of the GSP1/PRP20/YRB1 components of Candida albicans. Canadian Society of Microbiologists. Montréal, Canada. 1999.

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K. Julien, M. Tabrizian, R. Marchand, **P. Belhumeur** and L. Yahia. Nouveaux défis dans la stérilization des dispositifs médicaux: destruction des prions. ACFAS 67e congrès. Ottawa, Canada. 1999.

K. Julien, M. Tabrizian, R. Marchand, **P. Belhumeur** and L. Yahia. Approaches to Sterilization of Prion-Like Protcins. Annual Meeting, Society For Biomaterials. Providence, USA. 1999. (Trans Soc Biomater 1999: 562)

K. Julien, M. Tabrizian, R. Marchand, **P. Belhumeur** and L. Yahia. Novel sterilization techniques for prion-like proteins. Canadian Biomaterials Society. Québec, Canada. 1999.

N. Dubois, A.-R. Colina, F. Aumont, **P. Belhumeur** and L. de Repentigny. SAP2 constitutive overexpression expression and virulence of Candida albicans. ASM 98th General Meeting. Atlanta, USA.1998.

F. Deshaies et **P. Belhumeur**. Analyse structuro-fonctionnelle et génétique de LRE1, suppresseur multicopie d'un allèle mutant de GSP1 chez Saccharomyces cerevisiae. ACFAS 66e congrès. Québec, Canada. 1998.

M. Clément, H. Fournier, L. de Repentigny et **P. Belhumeur**. Instabilité chromosomique de mutants Gsp1: interaction entre Gsp1p et Sir4p. ACFAS 66e congrès. Québec, Canada. 1998.

M. Clément, H. Fournier, L. de Repentigny et **P. Belhumeur**. Clonage et caractérisation de GSP1 chez Candida albicans. ACFAS 65e congrès. Trois-Rivières, Canada.1997.

F. Deshaies, G. Barbès-Morin et **P. Belhumeur**. Analyse structuro-fonctionnelle de BOL1 chez Saccharomyces cerevisiae. ACFAS 65e congrès. Trois-Rivières, Canada. 1997.

N. Dubois, A.-R. Colina, F. Aumont, **P. Belhumeur** and L. de Repentigny. Expression of SAP2 in Saccharomyces cerevisiae and overexpression in Candida albicans. 97th ASM General Meeting. Miami, USA. 1997.

F. Aumont, A.-R. Colina, **P. Belhumeur** and L. de Repentigny. *Candida albicans* - Mucin-buccal epithelial cells interactions in vitro. 13th Congress of the International Society for Human and Animal Mycology. Parme, Italie. 1997.

N. Dubois, A.-R. Colina, F. Aumont, **P. Belhumeur** et L. de Repentigny. Clonage et expression hétérologue de la protéase *SAP2* de *Candida albicans* chez *Saccharomyces cerevisiae*. ACFAS 64e congrès. Montréal, Canada.1996.

M. Héjazi, W. Barth, F. Fortin, U. Stochaj et **P. Belhumeur**. Identification du signal de localisation nucléaire de la petite protéine G nucléaire *GSP1* chez *S. cerevisiae*. ACFAS 64e congrès. Montréal, Canada. 1996.

G. Barbès-Morin, F. Fortin, N. Desrochers et **P. Belhumeur**. *YCL051*, suppresseur génétique d'un mutant de *GSP1* chez *Saccharomyces cerevisiae*. ACFAS 64e congrès. Montréal, Canada. 1996.

A.R. Colina, F. Aumont, N. Deslauriers, **P. Belhumeur** and L. de Repentigny. Characterization of Candida albicans secretory mucinolytic activity. ASM Conference on Candida and Candidiasis. San Diego, USA. 1996.

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P. Belhumeur, J. Lanoix-Bergeron and D. Skup. Controlled formation of dsRNA and involvement of IFN in the differentiation of P19 embryonal carcinoma cells. Annual Meeting of the ISIR; Florence, Italy. 1989.

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G. Fleming, **P. Belhumeur**, D. Skup, D. and H.M. Fried. Functional substitution of yeast ribosomal protein L29 by mouse ribosomal protein L27' in yeast ribosome. Ribosome Synthesis Meeting; Cold Spring Harbor; USA. 1988.

P. Belhumeur and D. Skup. Expression of an antisense transcript to a ribosomal protein gene in murine cells. Great Lakes Mammalian Development Meeting; Toronto, Canada. 1987.
P. Belhumeur, G.D. Paterno and D. Skup. Isolation of cDNA clones differentially expressed in F9 embryonal carcinoma cells. Meeting "Genetics of Development"; Honey Harbor, Canada. 1985.

P. Belhumeur, G. Noël and G.R. Drapeau. Importance of SfiB protein in the control of cell division in *Escherichia coli*. Meeting of the Federation of European Microbiological Societies; Marseille, France. 1984.

J. Bergeron, **P. Belhumeur** and R. Letarte. Acquisition of ampicillin resistance by *Escherichia coli* in the fecal flora of Canadians travelling abroad. Annual Meeting of the American Society for Microbiology; Atlanta, USA. 1982.

Section V- Patents

P. Belhumeur, K. Julien, M. Tabrizian, L. Yahia, R. Marchand
Biological Indicators for validating a prion sterilization process.
International patent application No. PCT/CA00/00446 published under No. WO 00/65344 on November 2, 2000 resulting in National Phase entry applications in Brazil, Canada, Japan, Korea and the United States; and patents issued in Europe (validated in 15 countries), Australia, New Zealand and South Africa.

M. Lange, M. Clément, J. Tremblay, J. Thibodeau, P. Belhumeur
Method of suppressing the germination of Candida albicans using whey derived free fatty acids.
(Canadian patent application no. 2,464,777, filed on April 15, 2004)

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Antimicrobial agents derived from cream. (Canadian patent application no. 2,561,104, filed on September 26, 2006; United States patent application no. 11/580,633, filed on October 13, 2006)

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Free fatty acids for interfering with growth of Fusarium graminearum.
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Review

Similar and divergent features in mammalian and yeast prions

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Abstract

Mammalian transmissible spongiform encephalopathies are likely due to the propagation of an abnormal form of a constitutive protein instead of traditional genetic material (nucleic acids). Such infectious proteins, which are termed prions, exist in yeast. They are at the origin of a number of phenotypes that are inherited in a non-Mendelian manner. These prions are very useful to dissect the molecular events at the origin of this structure-based inheritance. The properties of mammalian and yeast prions are presented and compared. This review highlights a number of similarities and differences. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Prion; Amyloid fibrils; PrP; Sup35p; Ure2p; *Saccharomyces cerevisiae*; Auto-assembly; Nucleation-polymerization; Structural inheritance; Conformational changes; Molecular chaperones

1. Introduction

The idea that proteins could behave as infectious agents carrying alone the hereditary information that ensures their propagation emerged as early as 1967 [1,2]. This concept which contradicts the dogma that only nucleic acids could act as heritable elements was proposed to account for the considerable resistance to inactivation of a class of infectious agents made largely of proteins [3]. To explain the protein-based infectivity, it was proposed that proteins with such capacities are constitutive proteins that are able to exist in two different forms, one of which can catalyze the conversion of the normal form into the infectious one.

2. Mammalian prions

In mammals, the infectious protein (prion) at the origin of transmissible spongiform encephalopathies (TSEs) is believed to be a misfolded form of the *PRNP* gene product [4]. In healthy individuals the constitutive form of the prion protein PrP^c, a glycosylphosphatidyl-inositol-linked cell surface glycoprotein is protease-sensitive and is mainly expressed in the central nervous system in lymphatic tissues and at the neuromuscular junction. PrP^c has a turnover half-life of about 3 h [5] and appears not to be essential. Indeed, PrP-deficient mice are viable and can develop either normally or with minor defects [6]. However, mice expressing a truncated version of PrP^c in a null background show neuronal degeneration, suggesting that the protein may be involved in the maintenance and/or regulation of neuronal functions [7]. Recent data strongly suggest that PrP^c is a signaling molecule [8]. PrP^c would activate the tyrosine kinase Fyn, a member of a signal transduction cascade involved in neuronal cell maturation.

In individuals developing TSEs, PrP becomes protease-resistant (PrP^{sc} or PrP-res) [9]. PrP^c and PrP-res have the same chemical composition [10]. They differ in their secondary structure content [11]. PrP^c has a high α-helical content (40%) with no β-sheets, while the β-sheets and α-helical contents of PrP-res are 50% and 20%, respectively [11–13]. This together with a number of genetic studies (reviewed in [14,15]) has led to the view that TSEs are due to a change in the conformation of PrP^c. This change leads to the aggregation of PrP into stable high-molecular-weight oligomers that are resistant to proteinase K treatment and capable of converting PrP^c into PrP-res [16,17]. These aggregates, when purified by differential centrifugation following detergent solubilization, mainly consist of flattened rods [18]. There is no clear evidence that these aggregates constitute the self-propagating agent since they appear to be either poorly or not infectious. The picture becomes puzzling when data from a number of groups indicating that infectivity can be separated from fibrils and

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PrP-res [19–21] are taken into account. Thus, despite considerable investigations on the nature of the agent that causes TSEs and its propagation mechanism, a number of mysteries remain to be clarified.

3. Yeast prions

In yeast, genetic elements exhibiting aberrant properties have been described since the 1960s [22,23]. These elements produce phenotypic traits that are dominant, heritable in a non-Mendelian manner and transmissible by cytoduction [24]. These phenotypes are [PSI$^+$] and [URE3]. Their patterns of inheritance could readily be explained if the genes encoding these traits were non-chromosomal, i.e. mitochondrial, plasmidic or viral. But the genes in question have been mapped. They are chromosomal [25,26]. Some mutations in these genes mimic the [PSI$^+$] and [URE3] phenotypes given that these phenotypes are similar to mutations in *SUP35* and *URE2* genes that lead to the loss of function of these gene products. Surprisingly, however, these phenotypes occur with a frequency that is two orders of magnitude higher than that of point mutations. In addition, they can be lost when yeast cells are grown in the presence of millimolar concentrations of the protein denaturant guanidinium chloride [27,28] and can reappear without introduction of new DNA upon over-expression of *SUP35* and *URE2* gene products [28,29]. The [PSI$^+$] and [URE3] phenotypes are associated with Sup35p and Ure2p, respectively. Sup35p is an essential component of the translation termination machinery [30,31], while Ure2p acts as a negative regulator of nitrogen metabolism [32–34]. The loss of the function of Sup35p reduces the fidelity with which ribosomes terminate translation at stop codons, while that of Ure2p prevents the nitrogen-dependent repression of the ureidosuccinate utilization metabolism.

By analogy with the behavior of the mammalian prion protein PrP, it was proposed that the unusual inheritance of [PSI$^+$] and [URE3] would be a consequence of a self-perpetuating change in the conformation of *SUP35* and *URE2* gene products [28]. In other words, Sup35p and Ure2p would switch alternative conformational and functional states by the use of a prion-like autocatalytic process.

4. Model for prion propagation

The mechanism of prion propagation is still not fully understood at the molecular level. A number of models have been proposed [35–38]. The model that accounts for most of the events that characterize the propagation of prion disease is shown in Fig. 1. In this model, the native prion molecule P^c is in equilibrium with a conformational isoform P^{res}. P^{res} is rare and unstable in its monomeric state. It can be stabilized by complementary association with another molecule of P^{res} or can promote the conversion of either P^c or

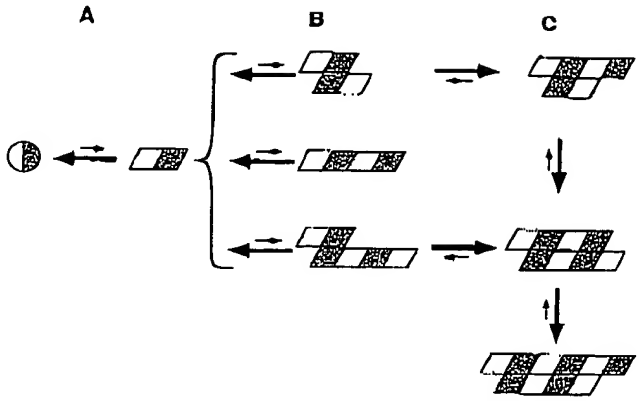

Fig. 1. Polymerization model for prion propagation. (A) The normal form of the prion protein is in equilibrium with the rare abnormal form of the prion protein that is the precursor of the aggregated form. (B) Prion proteins in their abnormal form can interact with each other. The interaction is unstable because the intermolecular interactions are not strong enough to outweigh the entropic cost of binding. Thus, the low-molecular-weight oligomers that are formed dissociate until a stable nucleus is formed. (C) This nucleus or seed can grow indefinitely from one or both ends. It can also break into smaller stable fibrils that can elongate by incorporation of the abnormal form of the prion protein.

one of its folding intermediates into P^{res} by a conformation rearrangement of P^c molecule or its folding intermediates. Since P^{res} is unstable its concentration must be very low and the formation of low-molecular-weight oligomers of P^{res} is not favored because the energy gained from intermolecular interactions does not outweigh the entropic cost of binding until a stable nucleus is formed. A number of inherited mutations that destabilize P^c predispose it to conversion to P^{res} [39], thus increasing the concentration of the latter form of the protein P^{res} and favoring its oligomerization. Once stable oligomers are formed, they can grow by incorporation of P^{res} at their ends. Such polymers can break into smaller units, each of which would behave as a seed. The requirement for stable nuclei to form before conversion is stable accounts for the low frequency of occurrence of prion diseases. However, the high efficiency of incorporation of P^c into P^{res} polymers accelerates prion propagation justifying a contact-based mode of transmission evoked in iatrogenic or dietary infections. A detailed mathematical model for prion propagation by nucleated polymerization has been developed and its parameters estimated from published data on PrP^{sc} propagation [40]. The model predicts kinetic aspects of the disease thus strengthening the nucleated polymerization hypothesis.

5. Putative function of prion proteins in their protease-resistant form

It is not trivial to imagine a useful function for the conformational change that leads to the abnormal protease-resistant state of proteins with prion properties, in particular

mammalian prions. A plausible function for the behavior of yeast prions has been proposed [41,42]. These epigenetic and metastable traits would provide a selective advantage for yeast cells in fluctuating environments. The phenotypes [URE3] and [PSI+] mimic the loss of function of Ure2p and Sup35p, respectively. However, because the genes of these proteins are present in an unaltered form in the cell, the loss of these functions can be considered reversible provided that Ure2p and Sup35p nuclei that perpetuate the structure-based mode of inheritance can be eliminated, counterbalanced or neutralized. Thus, prions might constitute a class of proteins whose activity can be modulated without requirement for genetic mutations. Such a property is of great interest in the field of protein engineering given that it could be used as a means to create heritable and adjustable changes in phenotype.

Sup35p inactivation results in an enhanced suppression of non-sense codons and non-sense codon mutations, i.e. a reduction in translational fidelity. In [PSI+]-mediated read-through, some naturally occurring stop codons or open reading frames that have acquired inactivating stop codon mutations may alter the function or the stability of encoded proteins or allow the expression of proteins that are not naturally expressed. Since the 3' untranslated regions of most genes are under fewer constraints than coding sequences they are highly polymorphic. The same applies for open reading frames in which inactivating stop codons have occurred. The [PSI+]-mediated expression of some of these proteins may contribute to phenotypic changes. If the new phenotype is advantageous, the population will grow and mutations will arise to fix the trait by eliminating stop codons that are relevant to the phenotype and require [PSI+] for read-through. Thus, [PSI+] phenotype, beside being a source of diversity, could facilitate the establishment of new traits, and allow the cell to occupy new niches.

6. Common structural features in mammalian and yeast prions

6.1. Primary structure

The primary structures of the yeast prions Sup35p and Ure2p and the mammalian prion PrP are unrelated. Nonetheless, prions share a striking feature. They all possess an N-terminally located prion-forming domain (PrD). This domain is not essential for protein function per se and has an unusual amino acid composition. The PrD of mammalian PrP (amino acid residues 23–88) has five complete octapeptide repeats of PHGGGWGQ. Although important, it is non-essential for prion disease propagation [43] suggesting that other features of PrP may be equally important. Indeed, a 106 polypeptide made of PrP amino acid residues 89–140 and 177–231 appears to be all that is required to confer susceptibility to and propagation of TSEs in vivo [44].

The PrD of Sup35p (amino acid residues 1–123) also has five or more imperfect oligopetiptide repeats of PQG-GYQQYN that resembles that of PrP. Both the number and primary sequence of these repeats play a key role in [PSI+] propagation [45–47]. In contrast with PrP and Sup35p, the PrD of Ure2p (amino acid residues 1–93) contains no repeats. However, the PrDs of Sup35p and Ure2p are similar in that they are particularly rich in Q and N residues while PrP is rich in neither amino acid. The Q- and N-rich regions of Sup35p and Ure2p are major contributors to the prion properties of these proteins [48]. Such regions may characterize yeast prions. Surveys of *Saccharomyces cerevisiae* proteome have revealed several proteins with Q- and N-rich domains [49,50]. There are indirect indications that these proteins, Rnq1p, New1p and Hrp1p, have prion-like properties. It is important to note that all proteins with Q- or N-rich domains are not necessarily prions. The protein huntingtin that is associated with amyloid-forming Huntington disease best illustrates this fact. Huntingtin has a poly-Q expansion; there is however no evidence that huntingtin amyloids are infectious [51].

Finally, the PrDs of yeast prions are modular and transferable by fusion to a number of proteins [41,49]. The resulting fusion proteins, when expressed in yeast cells, exist in distinct stable and heritable functional states.

6.2. Tertiary structures of mammalian and yeast prions

PrP, Sup35p and Ure2p have a common architecture. They are all two-domain proteins. The N-terminal domains are always poorly structured while the C-terminal domains are compactly folded [52–55].

The three-dimensional structure of recombinant PrPs extending from residues 90–231 of various species has been determined by NMR spectroscopy [52,53,56–62]. The COOH-terminal part of the molecule (residues 113–231) is compactly folded. It is made of three α-helices and a short antiparallel two-stranded β-sheet. This part of PrP exhibits two disparate surfaces. One is overall electrostatically positive but contains intermingled hydrophobic patches, suggesting that it could face the cell membrane. The opposite surface contains the two glycosylation sites and is electrostatically negative. This surface could interact with partner proteins or ligands. The N-terminal part of PrPs of various species (residues 23/29–112) lacks identifiable secondary structure under the experimental conditions used. The secondary and tertiary structures of this highly flexible region are of considerable interest because it is believed that profound conformational changes occur within this region during the formation of PrP^{sc}. Beside these major features, substantial differences are found mainly in various loops in PrPs from various species. These differences together with the homology between heterologous PrPs and the stability of PrP in recipient animals are believed to be important for the species barrier [63–65].

The three-dimensional structure of the structured part of the yeast prion Ure2p, extending from residue 95 to 354, has been solved recently by protein crystallography [66]. The protein is compactly folded into two domains. An N-terminal domain made of a four-stranded β-sheet flanked on each side by two α-helices and a C-terminal, all α-helical, domain. Ure2p is dimeric in its crystal form as well as in solution [67,68].

Ure2p95-354 shares a low degree of sequence identity with a class of enzymes involved in detoxification, the glutathione *S*-transferases [34]. A structural comparison reveals they have nevertheless a similar fold [66]. Ure2p differs from GSTs by its 90 N-terminal amino acid residues and a 32-amino acid residue insertion that is highly flexible since it can move by up to 6.2 Å and rotate by about 25° [66]. This region from one monomer is in the vicinity of the 90 N-terminal amino acid residues from the other monomer within the dimer, which suggests that they could interact. Such interaction could modulate the flexibility of the N-terminal region of the protein and its assembly properties.

6.3. Conversion of the constitutive form of prion proteins to other forms

Monomeric PrPs adopt alternative conformational states depending on solvent, pH and redox potential. At physiological pH, recombinant PrPs are mainly α-helical. The protein can fold to a soluble monomer composed almost entirely of β-sheets when the pH is adjusted to 4.0 [69,70]. This β-sheet state is prone to aggregation into fibrils.

Ure2p and Sup35p are also mainly α-helical. Although indirect evidence of the existence of pH-dependent conformational states of Ure2p that are the precursors of amyloid fibrils exists [67], there is no evidence of an overall alteration in the conformation of Ure2p and Sup35p. Indeed, while the structure of the N-terminal PrDs of yeast prions probably acquires an entirely β-sheet-rich conformation [71] that facilitates the interaction with molecules of the same type, the conformation of Ure2p and Sup35p C-terminal domains, amino acid residues 94–354 and 124–686, respectively, is believed to be unaffected. Thus, the inactivation of these proteins is believed to be a consequence of their sequestration from their normal associations.

6.4. Assembly properties

Prion diseases are associated with amyloidoses. However, about 20 human diseases are due to amyloid deposits of proteins that are not infectious [72]. Amyloids are fibrillar, detergent-resistant, high β-sheet protein polymers that stain with Congo red dye to give yellow–green birefringence under polarized light.

Most, if not all, polypeptides are capable of forming high β-sheet aggregates in suitable experimental conditions [73]. It is widely believed that the form of a given protein that assembles into amyloid fibers is a partially unfolded polypeptide chain. The fact that only a subset of proteins has the capacity to form such polymers has been attributed to an insufficient amount of folding intermediate that plays the role of amyloid precursor [73].

Ure2p and Sup35p spontaneously form amyloid fibrils in solution under physiological conditions [55,74–76]. The formation of these fibrils is greatly accelerated by seeding with preformed fibrils [55,74]. Furthermore, there is evidence that in vitro assembled fibrils made of recombinant Sup35p are infectious since when introduced in yeast cells they induce the de novo appearance of the prion phenotype [76]. Recombinant PrP aggregates in vitro into amyloid fibrils under acidic pH conditions and in the presence of protein denaturants [77,78]. However, such preformed fibrils do not induce the aggregation of PrP under neutral conditions and in the absence of protein denaturants, suggesting that partially unfolded intermediates of PrP are the precursors of PrP amyloids. In contrast with what is observed with yeast prions, such in vitro made fibrils are not infectious while the scrapie-associated aggregates are and induce, although in a sub-stoichiometric manner, the cell-free conversion of PrP^{c} to its scrapie-associated form. The differences in the behavior of yeast and mammalian prions reflect either different intrinsic properties of the proteins purely due to experimental situations or fundamental differences in the molecular basis of yeast versus mammalian prion propagation. Amyloid fibril formation can be accelerated in many cases by breaking preformed fibrils into smaller fibrils that can continue to grow. This is indeed what is observed when preformed Ure2p fibrils (L. Bousset, R. Melki, unpublished observations) or PrP fibrils [79] are sheared under experimental conditions where fibrils elongate. Thus, it is probable that mammalian and yeast prions propagate following similar molecular mechanisms.

6.5. Resistance to proteolysis

PrP^{c}, that has an apparent molecular mass of 33–35 kDa is completely degraded when limited proteinase K digestion is carried out on healthy animal brain and visceral tissue extracts. In contrast, PrP^{sc} is only partially cleaved in infected brain extracts subjected to the same treatment. The 66 N-terminal amino acids are removed yielding a species that has a molecular mass of 27–30 kDa. This differential resistance to proteolysis is relied on to allow the detection of PrP^{sc} in situ in both experimental and clinical diagnosis [80].

Such an increase in resistance to proteolysis has been reproduced in vitro [77] by incubation of protease-sensitive recombinant PrP under experimental conditions where an α-helix to β-sheet transition occurs accompanied by the formation of oligomers with fibrillar morphology (acidic conditions and the presence of guanidinium hydrochloride). Thus, it is tempting to propose that PrP^{sc} increased resis

tance to proteolysis is the result of a conformational transition that is similar to the one occurring in vitro.

Comparison of the proteinase K degradation patterns of Ure2p and Sup35p in wild-type yeast strains and strains exhibiting [URE3] or [PSI+] phenotypes reveals an unusual protease resistance in the latter strains [81–83]. Yeast prions are not as resistant to proteolysis as PrP^{sc} in cell extracts. Full-length Ure2p in its fibrillar form and its degradation products that are generated by proteinase K treatment are degraded within 5 min into polypeptides that have low molecular weight while PrPsc and its degradation products resist the same treatment for over 60 min. In addition, the degradation patterns of pure Ure2p in its soluble and aggregated forms show no significant differences in the polypeptide species generated [55]. The difference in the proteolytic patterns of the soluble and assembled forms of Ure2p resides in the time course of cleavage [55]. This suggests that the conformation of the C-terminal region of Ure2p is not altered upon assembly, since the fragments produced have been attributed to the C-terminal domain of the protein [67].

6.6. Prion strains and species barrier

Two intriguing features characterize prion propagation: (i) clinically different prion disease can occur within one animal species without variation in PrP genotype; and (ii) prions propagate between species much less efficiently than within a given species.

The heritable variation in the clinical presentation and detailed neuropathology or phenotypic traits is at the origin of the classification of prion diseases into various strains. Strain differences in mammalian [84] and yeast [85] prion structure and conformation have been described. Strain propagation is attributed to conformational templating or seeding mechanisms [86]. Recent evidence for a link between the prion's conformation and seeding specificity has been shown [87]. Using the PrDs of Sup35p from *Candida albicans*, *S. cerevisiae* and a chimaeric PrD in which both domains are present, it was shown that, depending on the species from which seeding fibers originate, the chimaeric PrD can fold into two different conformations, themselves with distinct in vitro seeding specificities [87]. This work has established that the prion's primary structure dictates the spectrum of favoured strain conformations. It also suggests that prion conformation may restrict the transmission of prion diseases between species. However, given that distinct prion strains within one species may be caused by differing prion conformations, some conformations may have an enhanced ability to cross the species barrier. Thus, the species barrier and strain appear to be intimately related phenomena. Indeed, the observation that the efficiency of a cell-free conversion reaction between heterologous PrP-res and PrP-sen molecules correlates with the transmissibility of the disease between species and strains of species suggests that sequence homology affects direct interactions between PrP-res and PrP-sen molecules [64,65,88]. The same conclusion is reached in the transmission of [PSI+] phenotype where the species barrier effects are determined by the PrD of various Sup35p molecules [50,89,90].

7. Features that differ in mammalian and yeast prions

7.1. Intrinsic properties and cellular activities

The primary structures of mammalian and yeast prions are unrelated. Little is known about their activities but they differ significantly. They bind various ligands. PrP binds metal ions [91–95], Ure2p binds glutathione [96] and Sup35p binds GTP [97]. Binding of copper to PrP induces a change in its conformational state [93,94]. It is not clear whether binding of ligands to yeast prions modifies their assembly properties.

Yeast prions do not spread from cell to cell and do not usually kill the cells harbouring them. They are inherited by daughter cells from mothers or passed between partners during mating. In contrast, neuronal death is observed in animals expressing PrP^{c} infected by PrP-res. A number of observations suggest however that PrP-res is not, by itself, cytotoxic. Indeed, when neuroectodermal tissues from mice expressing PrP^{c} are grafted into the brains of *Prn-p*$^{0/0}$ mice, no detectable pathology is observed in the host tissue after administration of PrP^{sc}, its accumulation in the graft and even its migration from the graft into the host tissue [98,99].

7.2. The mechanism of conversion of mammalian and yeast prions into their infectious forms differ significantly

Unlike yeast prions, PrP is not a cytoplasmic protein. It is a glycoprotein attached to the membrane via a GPI-anchor that transits through secretory then degradation pathways. It is therefore very likely that the formation of the infectious form of PrP occurs after it transits into a subcellular compartment such as the lysosome, i.e. that the formation of infectious PrP occurs in a molecular environment that is very different from the cytoplasmic environment. This view is reinforced by the findings that distinct equilibrium unfolding intermediates of PrPs, with a predominantly β-sheet topology, are observed at pH 4.0 [69,100]. The change in the conformation of yeast prions is believed to occur at physiological pH in the cytoplasm [101].

Just as mammalian PrP is prone to aggregation into large aggregates in the brain of infected animals [16], Sup35p and Ure2p are found in large aggregates in [PSI+] and [URE3] strains [81,82,102]. However, PrP deposits, when treated by detergents, appear to be made of PrP fibrils, while there is no conclusive evidence that the aggregated form of Sup35p and Ure2p in yeast cells is made of fibrils.

7.3. Role of molecular chaperones

The molecular chaperone HSP104 plays a crucial role in the propagation of [PSI+] phenotype [103]. HSP104 is believed to influence Sup35p assembly by fragmentation of Sup35p aggregates [104]. Whether the same molecular chaperone influences the formation and maintenance of [URE3] phenotype is still an open question. No mammalian HSP104 homolog has been reported nor would it be expected to be present in the cellular compartment to which PrP is delivered. Recent data have demonstrated that mouse PrP expressed in yeast cytoplasm acquires the physicochemical characteristics of PrP-res [105]. No effect of endogenous HSP104 on the conformation of PrP is reported in this work. Instead, the importance of the oxidative–reductive balances and that of the glycosylation status of PrP on its conformational transitions are underlined.

7.4. Yeast prions meet genetic criteria while mammalian prions do not

Three genetic criteria need to be fulfilled in order to establish that an infectious agent is a prion and not a virus, plasmid or other nucleic acid replicon: (i) reversible curability; (ii) induction of prion formation by over-expression of the normal protein; and (iii) a unique phenotypic relationship between the prion state and mutations in the gene of the protein [28].

None of the mammalian prions satisfy these genetic criteria. It is therefore not yet proven that the infectious agent at the origin of the TSEs is indeed a prion. PrP clearly plays a central role in these diseases, but proof that it is identical to the infectious agent is still missing.

8. Conclusion

Important steps have been made in understanding prion diseases at the structural level. This includes the elucidation of the structure and the folding dynamics of PrP^{c} and the characterization of the state of the protein (PrP-res) that is associated with infectivity. The demonstration that PrP^{c} can be recruited by PrP^{sc} aggregates and imprinted by the parental PrP^{sc} conformation has opened the way for a better understanding of the molecular events at the origin of prion propagation and the strain aspect associated with prion diseases. Biochemical and structural studies have narrowed down the region of PrP necessary for prion propagation to 106 amino acid residues. Nevertheless, no one has yet produced infective prions in vitro from purified recombinant PrP. Such an achievement would not only prove the protein-only hypothesis in mammalian TSEs but also serve to understand the molecular details of PrP^{sc} formation and propagation.

Yeast genetic methods have provided evidence of infectious proteins. Genetic criteria have been established to define that an infectious agent is a prion. These criteria have been exploited to identify new prions of yeast and other organisms. Four potential prions were discovered with this approach. Three in the yeast *S. cerevisiae*, Rnq1p [49], Hrp1p [49] and New1p [50], and one in the filamentous fungus *Podospora anserina*, Het-s protein [106].

Table 1
Comparison of the genetic, biochemical and structural properties of mammalian and yeast prions

Trait	PrP	Sup35p	Ure2p
Phenotype relation	no	yes [a]	yes [b]
Reversible curability	no	yes	yes
Prion induction by over-expression	no	yes	yes
Localization	membranes	cytosol	cytosol
Propagation			
In vivo	yes	yes [c]	yes [c]
In vitro	yes	yes	yes
Infectious protein aggregates	yes	yes	yes
Protein domains	≥ 2	≥ 2	≥ 2
Amino acid repeats	yes [d]	yes [e]	yes [f]
Poorly structured N-terminal domain	yes	yes	yes
Compactly folded C-terminal domain	yes	yes	yes
Tertiary structure	yes [g]	no	yes [g]
Quaternary structure	monomer [h]	unclear	dimer
Soluble oligomers	yes	yes	yes
Insoluble oligomers	yes	yes	yes
Amyloid fibrils	yes	yes	yes
Change in resistance to proteolysis	yes	yes	yes
Seeded assembly	no [i]	yes	yes
Prion strains	yes	yes	no [i]
Species barrier	yes	yes	no [i]

[a] [PSI+] phenotype.
[b] [URE3] phenotype.
[c] Mother-to-daughter inheritance or propagation during mating.
[d] Five octapeptide repeats of PHGGGWGQ.
[e] At least five repeats of the consensus oligopeptide PQGGYQQYN. Very rich in Q.
[f] Very rich in N and Q.
[g] 3D-NMR or crystal structures of only the compactly folded domain available.
[h] Dimeric forms have been reported in solution and in crystals [110].
[i] Not demonstrated.

Biochemical analysis has shown that much like PrP, Ure2p and Sup35p can exist in two states. The assembly properties of these proteins are actively examined and their PrDs used to document two crucial aspects of prion propagation: (i) the species barrier, and (ii) the strain phenomena.

These studies have added significantly to our understanding of the molecular basis of yeast prion conversion and propagation. However, though mammalian and yeast prions share similar traits, they differ in a number of features. The similarities and differences are summarized in Table 1. Thus, caution must be taken in directly extrapolating findings with yeast prions to mammalian prions.

The striking capacity of prions to self-promote structural changes that are reversible at least in yeast cells raises the question about their utility in vivo. Given that the genes for these proteins are unaltered and the loss of the function of prion proteins reversible, it was proposed that the behavior of prion proteins provides a means of modulating protein

activity without requiring genetic mutations [107,108], in other words a novel mechanism for environmental adaptation that uses the metastable state of functional proteins and/or preadaptation to different selective niches [108]. While this may well be the case for [URE3] and [PSI+] phenotypes, these ideas are difficult to reconcile with TSEs and [Het-s] phenotype.

Much has been learned about the biology of both mammalian and yeast prions during the last decade. It is time to use this knowledge to develop therapeutic strategies for TSEs and amyloid diseases with approaches such as structure-based drug design [109].

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